Promega

Technical Bulletin

pGEM®-3Z Vector

INSTRUCTIONS FOR USE OF PRODUCT P2151.

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Part# TB033

pGEM®-3Z Vector

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1. Description

The pGEM®-3Z Vector is intended for use as a standard cloning vector, as well as for the highly efficient synthesis of RNA in vitro. The vector carries the *lac*Z α-peptide and multiple cloning region arrangement from pUC18 (1). In addition, the vector contains both the SP6 and T7 RNA polymerase promoters flanking the multiple cloning region. This arrangement gives rise to a functional α-peptide that is capable of complementing the product of the *lac*ZΔM15 gene to produce functional β-galactosidase. Cells with the genotype, *lac*ZΔM15, and also containing the pGEM®-3Z Vector will be blue when plated on indicator media containing IPTG and X-Gal. However, when the *lac*Z α-peptide sequence is disrupted by cloning into the pGEM®-3Z multiple cloning region, complementation does not occur and no β-galactosidase activity is produced. Therefore, bacterial colonies harboring recombinant pGEM®-3Z Vector constructs remain white.

The sequences of Promega vectors are available online at: **www.promega.com/vectors/** and are also available from the GenBank® database.

2. Product Components and Storage Conditions

Product	Size	Cat.#
pGEM®-3Z Vector	20µg	P2151

Storage Conditions: Store the pGEM®-3Z Vector at –20°C.

3. pGEM®-3Z Vector Multiple Cloning Region and Circle Map

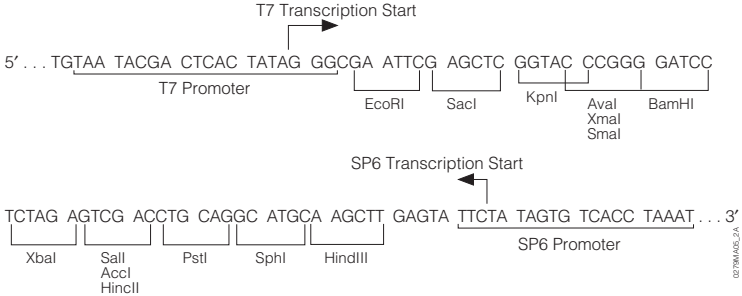

Figure 1. pGEM®-3Z Vector promoter and multiple cloning region sequence. The sequence shown corresponds to RNA synthesized by T7 RNA polymerase and is complementary to RNA synthesized by SP6 RNA polymerase.

Note: The pGEM®-3Z and pGEM®-4Z Vectors are identical except for the orientation of the SP6 and T7 promoters.

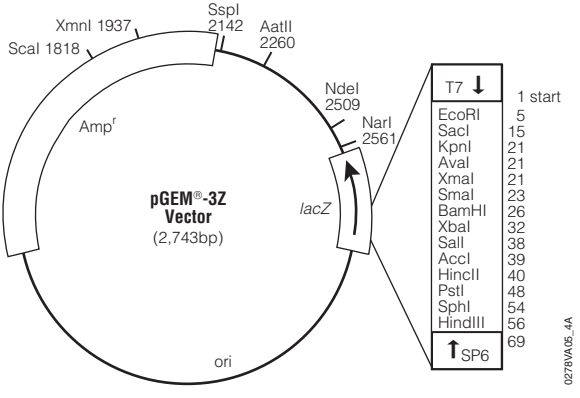

Figure 2. pGEM®-3Z Vector circle map and sequence reference points.

pGEM®-3Z Vector sequence reference points.	
T7 RNA polymerase transcription initiation site	1
multiple cloning region	5–61
SP6 RNA polymerase promoter (–17 to +3)	67–86
SP6 RNA polymerase transcription initiation site	69
lac operon sequences	94–323; 2564–2724
binding site of pUC/M13 Reverse Sequencing Primer	104–125
*lac*Z start codon	108
*lac*Z operator	128–144
β-lactamase (Amp^r) coding region	1265–2125
binding site of pUC/M13 Forward Sequencing Primer	2677–2700
T7 RNA polymerase promoter (–17 to +3)	2727–3

Specialized applications of the pGEM®-3Z Vector.

- Blue/white screening for recombinants.
- Transcription in vitro from dual, opposed promoters. (For protocol information, please see the *Riboprobe® in vitro Transcription Systems Technical Manual*, #TM016.)

4. pGEM®-3Z Vector Restriction Sites

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3´ end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes, or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. The vector sequence is available in the GenBank® database (GenBank®/EMBL Accession Number X65304) and on the Internet at: **www.promega.com/vectors/**

Table 1. Restriction Enzymes That Cut the pGEM®-3Z Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AatII	1	2260
AccI	1	39
Acc65I	1	17
AcyI	3	1875, 2257, 2561
AflIII	1	445
Alw26I	4	1399, 2175, 2328, 2370
Alw44I	3	759, 2005, 2502
AlwNI	1	861
AspHI	5	15, 763, 1924, 2009, 2506
AvaI	1	21
AvaII	2	1476, 1698
BamHI	1	26
BanI	4	17, 189, 1286, 2560
BanII	1	15
BbeI	1	2564
BbuI	1	54
BglI	2	1458, 2576
BsaI	1	1399
BsaOI	5	361, 785, 1708, 1857, 2604
BsaHI	3	1875, 2257, 2561
BsaJI	5	21, 22, 184, 605, 2679
Bsp1286I	5	15, 763, 1924, 2009, 2506
BspHI	3	1165, 2173, 2278
BspMI	1	51
BssSI	3	618, 2002, 2309
BstOI	5	185, 473, 594, 607, 2680
Cfr10I	1	1418
DraI	3	1204, 1223, 1915
DraII	1	2314
DrdI	2	553, 2422
EaeI	3	284, 1726, 2713
EarI	3	329, 2133, 2621
EclHKI	1	1338
EcoICRI	1	13
EcoRI	1	5
EheI	1	2562
FokI	5	1304, 1485, 1772, 2415, 2659
FspI	2	1560, 2583
HaeII	3	323, 693, 2564
HgaI	4	556, 1134, 1864, 2422
HincII	1	40
HindII	1	40
HindIII	1	56
Hsp92I	3	1875, 2257, 2561
KasI	1	2560
KpnI	1	21
MaeI	4	33, 940, 1193, 1528
MaeII	5	1148, 1564, 1937, 2257, 2699
NarI	1	2561
NdeI	1	2509
NspI	3	54, 449, 2366
PleI	5	44, 339, 824, 1327, 2727
PspAI	1	21
PstI	1	48
PvuI	2	1708, 2604
PvuII	2	269, 2633
RsaI	3	19, 1818, 2494
SacI	1	15
SalI	1	38
ScaI	1	1818
SinI	2	1476, 1698
SmaI	1	23
SphI	1	54
Sse8387I	1	48
SspI	1	2142
TaqI	4	9, 39, 545, 1989
TfiI	2	280, 420
VspI	3	216, 275, 1510
XbaI	1	32
XmaI	1	21
XmnI	1	1937

Note: The enzymes listed in boldface type are available from Promega.

Table 2. Restriction Enzymes That Do Not Cut the pGEM®-3Z Vector.

AccB7I	BsaBI	DraIII	**NgoMIV**	RsrII
AccIII	**BsaMI**	DsaI	**NheI**	**SacII**
AflII	BsmI	EagI	**NotI**	**SfiI**
AgeI	Bsp120I	**Eco47III**	**NruI**	**SgfI**
ApaI	BsrGI	**Eco52I**	**NsiI**	SgrAI
AscI	**BssHII**	Eco72I	PacI	**SnaBI**
AvrII	Bst1107I	Eco81I	PaeR7I	**SpeI**
BalI	**Bst98I**	EcoNI	PflMI	SplI
BbrPI	**BstEII**	**EcoRV**	PinAI	SrfI
BbsI	**BstXI**	FseI	PmeI	**StuI**
BclI	**BstZI**	**HpaI**	PmlI	**StyI**
BglII	**Bsu36I**	**I-PpoI**	Ppu10I	SwaI
BlpI	**ClaI**	**MluI**	PpuMI	**Tth111I**
Bpu1102I	**CspI**	**NaeI**	PshAI	XcmI
BsaAI	**Csp45I**	**NcoI**	Psp5II	**XhoI**

Table 3. Restriction Enzymes Cut the pGEM®-3Z Vector 6 or More Times.

AciI	**CfoI**	**HinfI**	MnlI	NlaIV
AluI	**DdeI**	**HpaII**	MseI	**Sau3AI**
BbvI	**DpnI**	HphI	**MspI**	Sau96I
BsrI	DpnII	**Hsp92II**	**MspA1I**	ScrFI
BsrSI	Fnu4HI	MaeIII	**NciI**	SfaNI
Bst71I	**HaeIII**	**MboI**	**NdeII**	**Tru9I**
BstUI	**HhaI**	**MboII**	NlaIII	**XhoII**

Note: The enzymes listed in boldface type are available from Promega.

5. Related Products

Product	Size	Cat.#
pGEM®-4Z Vector	20µg	P2161
pGEM®-3Zf(+) Vector	20µg	P2271
pGEM®-3Zf(–) Vector	20µg	P2261
pGEM®-5Zf(+) Vector	20µg	P2241
pGEM®-5Zf(–) Vector	20µg	P2351
pGEM®-7Zf(+) Vector	20µg	P2251
pGEM®-7Zf(–) Vector	20µg	P2371
pGEM®-9Zf(–) Vector	20µg	P2391
pGEM®-11Zf(+) Vector	20µg	P2411
pGEM®-11Zf(–) Vector	20µg	P2421
pGEM®-13Zf(+) Vector	20µg	P2541

Product	Size	Cat.#
pSP64 Poly(A) Vector	20µg	P1241
pSP72 Vector	20µg	P2191
pSP73 Vector	20µg	P2221

Sequencing Primers

Product	Size	Cat.#
SP6 Promoter Primer	2µg	Q5011
T7 Promoter Primer	2µg	Q5021
pUC/M13 Primer, Reverse (17mer)	2µg	Q5401
pUC/M13 Primer, Forward (17mer)	2µg	Q5391
pUC/M13 Primer, Forward (24mer)	2µg	Q5601
pUC/M13 Primer, Reverse (22mer)	2µg	Q5421

Riboprobe® in vitro Transcription Systems

Product	Cat.#
Riboprobe® System—SP6	P1420
Riboprobe® System—T7	P1440

For Laboratory Use.

RiboMAX™ Large Scale RNA Production Systems

Product	Cat.#
RiboMAX™ Large Scale RNA Production System—SP6	P1280
RiboMAX™ Large Scale RNA Production System—T7	P1300

For Laboratory Use.

6. Reference

1. Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) Improved M13 phage cloning vectors and host strains: Nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**, 103–19.